PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference PCT25791	**FOR FURTHER ACTION** See Form PCT/IPEA/416	
International application No. PCT/IT2004/000689	International filing date *(day/month/year)* 10.12.2004	Priority date *(day/month/year)* 11.12.2003

International Patent Classification (IPC) or national classification and IPC
INV. A61K31/4985 A61K31/505 A61K31/475 A61K38/12 A61K38/16 G01N33/574 C07K7/00 A61P35/04

Applicant
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1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 9 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 5 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☒ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in celectronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
10.10.2005	29.03.2006
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Bochelen, D Telephone No. +49 89 2399-8150

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/IT2004/000689

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:

   ☐ international search (under Rules 12.3 and 23.1(b))
   ☐ publication of the international application (under Rule 12.4)
   ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-5, 7-58	as originally filed
6	filed with telefax on 10.10.2005

Claims, Numbers

1-32	received on 18.10.2005 with letter of 18.10.2005

Drawings, Sheets

1/19-19/19	as originally filed

☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:

   ☐ the description, pages
   ☐ the claims, Nos.
   ☐ the drawings, sheets/figs
   ☐ the sequence listing *(specify)*:
   ☐ any table(s) related to sequence listing *(specify)*:

4. ☒ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

   ☐ the description, pages
   ☒ the claims, Nos. 1,5-6
   ☐ the drawings, sheets/figs
   ☐ the sequence listing *(specify)*:
   ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/IT2004/000689

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	3-4,11,31
	No:	Claims	1,5-6,10,22
Inventive step (IS)	Yes:	Claims	3-4
	No:	Claims	1-2,5-31
Industrial applicability (IA)	Yes:	Claims	1-31
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this report has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed

   ☒ filed together with the international application in computer readable form

   ☐ furnished subsequently to this Authority for the purposes of search and/or examination

   ☐ received by this Authority as an amendment on

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional observations, if necessary:

Re Item I
Basis of the report

1. The amendments filed with the letter dated 18.10.05 introduce subject-matter which extends beyond the content of the application as filed, contrary to Article 34(2)(b) PCT. The amendments concerned are the following: the restriction to a peptide **having** the amino acid sequence of SEQ ID No 9 in claims 1, 5 and 6. Throughout the original application were mentioned only peptides **comprising** an amino acid sequence of SEQ ID No 9.

Re Item V
Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

2. Prior art:

Reference is made to the following documents:

D1: US-B1-6 486 300 (BANDMAN OLGA ET AL) 26 November 2002 (2002-11-26)

D2: COLLIER G R ET AL: "INHIBITION OF LUNG METASTASIS FORMATION BY A RAT OSTEOGENIC SARCOMA SUBCLONE USING PYRIMIDO-PYRIMIDINE DERIVATIVES" AUSTRALIAN AND NEW ZEALAND JOURNAL OF MEDICINE, ROYAL AUSTRALASIAN COLLEGE OF PHYSICIANS, SYDNEY, AU, vol. 15, no. 1, SUPPL 1, February 1985 (1985-02), page 127, XP008046052 ISSN: 0004-8291

D3: BANDO H ET AL: "EFFECTS OF ANTIPLATELET AGENTS ON PULMONARY METASTASES" GANN, JAPANESE CANCER ASSOCIATION, TOKYO, JP, vol. 75, no. 3, March 1984 (1984-03), pages 284-291, XP009013087 ISSN: 0016-450X

D4: BERTRAM J S ET AL: "INHIBITION OF NEOPLASTIC CELL GROWTH BY QUIESCENT CELLS IS MEDIATED BY SERUM CONCENTRATION AND CYCLIC AMP PHOSPHO DI ESTERASE INHIBITORS" JOURNAL OF CELLULAR BIOCHEMISTRY, vol. 18, no. 4, 1982, pages 515-538, XP002329792 ISSN: 0730-2312

D5: NI XIAOHUA ET AL: "Isolation and characterization of a novel human NM23-H1B gene, a different transcript of NM23-H1." JOURNAL OF HUMAN

GENETICS, vol. 48, no. 2, February 2003 (2003-02), pages 96-100, XP002329793 ISSN: 1434-5161

D6: POSTEL EDITH H ET AL: "Mutational analysis of NM23-H2/NDP kinase identifies the structural domains critical to recognition of a c-myc regulatory element" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, vol. 93, no. 14, 1996, pages 6892-6897, XP002329794 ISSN: 0027-8424

D7: REYMOND ALEXANDRE ET AL: "Evidence for interaction between human PRUNE and nm23-H1 NDPKinase" ONCOGENE, vol. 18, no. 51, 2 December 1999 (1999-12-02), pages 7244-7252, XP002329795 ISSN: 0950-9232

D8: FORUS ANNE ET AL: "Amplification and overexpression of PRUNE in human sarcomas and breast carcinomas: A possible mechanism for altering the nm23-H1 activity" ONCOGENE, vol. 20, no. 47, 18 October 2001 (2001-10-18), pages 6881-6890, XP002329796 ISSN: 0950-9232

D9: ZOLLO M ET AL: "Prune and nm23-H1 and nm-23 H2 (NDP-Kinase) proteins: Involvement in cancer" AMERICAN JOURNAL OF HUMAN GENETICS, vol. 69, no. 4 Supplement, October 2001 (2001-10), page 273, XP009047948 & 51ST ANNUAL MEETING OF THE AMERICAN SOCIETY OF HUMAN GENETICS; SAN DIEGO, CALIFORNIA, USA; OCTOBER 12-16, 2001 ISSN: 0002-9297

D10: DATABASE Geneseq [Online] 26 June 2001 (2001-06-26), "Human cDNA clone (5'-primer) SEQ ID NO:5290." XP002329797 retrieved from EBI accession no. GSN:AAH08455 Database accession no. AAH08455

D11: DATABASE Geneseq [Online] 6 November 2003 (2003-11-06), "Human intracellular signalling molecule INTSIG-44, SEQ ID NO:44." XP002329798 retrieved from EBI accession no. GSN:ADA13362 Database accession no. ADA13362

D12: DANGELO A ET AL: "The human cyclic nucleotides phosphodiesterase (PDE) Prune protein: A dual cellular compartment localization and functional properties." AMERICAN JOURNAL OF HUMAN GENETICS, vol. 71, no. 4 Supplement, October 2002 (2002-10), page 513, XP002329885 & 52ND ANNUAL MEETING OF THE AMERICAN SOCIETY OF HUMAN GENETICS; BALTIMORE, MD, USA; OCTOBER 15-19, 2002 ISSN: 0002-9297

If not indicated otherwise the relevant passages are those mentioned in the

search report.

Document D1 discloses the use of human nm23, comprising a peptide sequence of SEQ ID No 9 of the application, for inhibiting metastasis.

Document D2 discloses the use of dipyridamole for inhibiting metastasis.

Document D3 discloses the inhibition of metastasis by dipyridamole

Document D4 discloses the inhibition of metastasis of Lewis lung carcinoma by the PDE inhibitor isobutyl-methylxanthine.

Document D5 discloses the sequence of nm23-H2, defined as a putative metastasis suppressor, which comprises a peptide of sequence of SEQ ID No 9 of the application.

Document D6 discloses the sequence of nm23, comprising an amino acid sequence of SEQ ID No 9 of the application, which is a presumed regulator of tumour metastasis.

Document D7 discloses that Prune interacts with nm23 and the uncoupling of this interaction might lead to neuroblastoma progression.

Document D8 discloses the over-expression and amplification of PRUNE assessed by immunohistochemistry, FISH and northern blot in tumours expressing nm23 and in metastasising tumours.

Document D9 discloses the interaction of the PDE Prune with the tumour metastasis inhibitor gene nm23-H1. Document D9 discloses that Prune is amplified in tumour cells as shown by FISH and immunohistochemistry.

Document D10 discloses a nucleic acid sequence comprising the sequence of SEQ ID No 1 of the application which is a 5'-primer.

Document D11 discloses a peptide comprising a sequence of SEQ ID No 4 of the application and antibodies specific for this peptide.

Document D12 discloses that the Prune protein possesses phosphodiesterase activity.

2. Novelty (Art. 33 (1) and (2) PCT):

2.1 Claim 1 is not novel over the disclosure of documents D1. Claim 1 is interpreted as relating to a peptide **comprising** a sequence of SED ID No 9 (see above point 1). Document D1 does not disclose that nm23, which comprises the amino acid sequence of SEQ ID No 9, is an inhibitor of the cyclic nucleotide phosphodiesterase activity of Prune, however D1 discloses the inhibition of metastasis by these peptides. The presence of a mechanism of action described in the application, i.e inhibition of Prune activity, cannot be used to delimit the present claims from the state of the art. The end effect of the presently claimed invention is the treatment of metastasis using the same peptide as disclosed in the prior art. The mechanism of action is therefore merely a discovery of how the peptide comprising the amino acid sequence of SEQ ID No 9 could work. Claim 1 does not fulfill the requirements of Art. 33(2) PCT.

2.2 Claims 5 and 6 are interpreted as relating to a peptide **comprising** a sequence of SED ID No 9 (see above point 1). Said claim is lacks thus novelty over D1 and D5-D6.

2.3 Claim 10 lacks novelty over document D8-D9 which discloses the increased expression of Prune in metastasising tumours (see p6882 col2 1st §). Claim 11 does not fulfill the requirements of Art. 33(2) PCT.

2.4 Claim 22 lacks novelty over documents D7-D9 which disclose the detection of PRUNE by FISH (D7: page 7246 col 1 §1; D8: p6887 col2; D9: abstract).

3. Inventive step (Art. 33 (1) and (3) PCT):

3.1 The peptide comprising a sequence of SEQ ID No 10 which is subject-matter of claims 3-4 is neither disclosed nor suggested in the prior art. Claims 3-4 fulfill the requirement of Art. 33(3) PCT. The use thereof for preventing metastasis would involve an inventive step.

3.2 Document D12 discloses that the Prune protein possesses phosphodiesterase

catalytic activity. The method of screening of claim 7 uses a specific cell line overexpressing h-PRUNE. However, it would be obvious for a skilled man to use a cell line overexpressing h-PRUNE in a method for screening inhibitors of phosphodiesterase activity. Furthermore, the use of the specific cell line of claim 7 does not result in an unexpected advantage over the prior art. Claim 7 does thus not fulfill the requirement of Art. 33(3) PCT.

3.3 Claim 11 differs from document D8 (see page 6888 col2 1st§) in that a monoclonal antibody is used. However, it would be obvious for a skilled to use a monoclonal antibody against PRUNE instead of a polyclonal antibody. Claim 11 does thus not fulfill the requirement of Art. 33(3) PCT.

3.4 Antibodies directed to Prune are known in the art (see D8: p6885 fig3). It would be obvious for a skilled man to produce an alternative monoclonal antibody specific for Prune. Claim 32 thus lacks inventive step in the sense of Art. 33(3) PCT.

3.5 Dependent claims 8-9, 13-21 and 23-31 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of inventive step for the following reasons:

Claim 2 is a selection of specific tumours which is not inventive in view of D1.

Claims 8-9 relate to specific conditions of screening which would be obvious in view of D12.

Claims 12-14 are obvious in view of D8. The use of an alternative monoclonal antibody is not inventive.

The use of specific primers or labelling in the methods of claims 12-21 and in the kit of claims 23-31 cannot be considered as involving an inventive step.

3.6 It is further noted that an inventive step would be acknowledged for the use according to claim 1 of IC261.